LETTER

REPLY TO HALANYCH ET AL.:

# Ctenophore misplacement is corroborated by independent datasets

Davide Pisani[a,b,1,2], Walker Pett[c,1], Martin Dohrmann[d], Roberto Feuda[e], Omar Rota-Stabelli[f], Hervé Philippe[g,h], Nicolas Lartillot[c], and Gert Wörheide[d,i,2]

In their letter, Halanych et al. (1) criticize our recent assertion (2) that the phylogenetic placement of ctenophores as the sister group to all other animals (the Ctenophora-sister hypothesis) in three previous studies (3–5) was an artifact caused by undetected systematic error.

Halanych et al. (1) claim we used no "objective approaches" to identify sources of systematic error. In fact, we used an objective comparison of Bayesian cross-validation scores to select the best-fitting substitution model, because poorly fitting models are a frequent source of systematic error. Halanych et al. point out that this comparison did not include partitioned site-homogeneous models. However, they do not mention that only one of the studies we address (3) used this approach, and that multiple site-homogeneous partitions still do not account for within-partition site-heterogeneous biochemical constraints, which our results show had a major impact on model fit and the tree topology.

Halanych et al. (1) also incorrectly suggest that our model-selection procedure relied on circular assumptions about the position of sponges. No such assumptions were made to demonstrate the better fit of site-heterogeneous models. In fact, our cross-validation tests were conservative in favor of Ctenophora-sister, because we "trained [all models] under the tree topology favored by WAG [Whelan and Goldman; Ctenophora-sister], thus making the test conservative in favor of the WAG model [and consequently Ctenophora-sister]" (*Methods* in ref. 2).

Halanych et al. (1) further claim that our conclusions rested extensively on unconverged analyses. In reality, our conclusions about the position of Ctenophora were based solely on converged analyses, with two unconverged analyses mentioned only for completeness in one case, or to demonstrate weak phylogenetic signal in the other (reanalysis of ref. 5).

The main criticism of Halanych et al. (1) is that we arbitrarily preferred results obtained using only closely related outgroups. More accurately, our results simply showed weaker average support for Ctenophora-sister under better-fitting models. When only holozoan (not just choanoflagellate) outgroups were included, support for Ctenophora-sister did deteriorate (figure 2 B and C in ref. 2), or support for Porifera-sister emerged (figure 2A in ref. 2), and this effect became more pronounced when using only choanoflagellates. This sensitivity of the position of Ctenophora to model choice after excluding distant outgroups suggests these outgroups exacerbate systematic error by obscuring the effect of better-fitting models.

The final comment in Halanych et al. (1) about the questionable use of gene content data is unjustified. The observation that "gene content varies greatly within phyla" (1) is evidence that it contains phylogenetic signal. Accordingly, analysis of gene content (corrected for ascertainment bias) recovered well-established bilaterian relationships (figure 3 in ref. 2), and corroborated nonbilaterian relationships inferred from amino acid data (figure 1 in ref. 2).

Finally, Halanych et al. (1) claim that our conclusions mistreated complex traits as single characters and ignored the position of Placozoa. Actually, we acknowledged this by stating "Future studies ... will help to clarify the relationship between the homology of similar structures and their underlying genetic mechanisms," and "resolving the exact phylogenetic positions of Ctenophora and Placozoa ... will be crucial to reconstruct the evolution of key characters, such as nervous systems, muscles, and digestive tracts, in more detail" (2).

[a]School of Earth Sciences, University of Bristol, Bristol BS8 1TG, United Kingdom; [b]School of Biological Sciences, University of Bristol, Bristol BS8 1TG, United Kingdom; [c]Laboratoire de Biométrie et Biologie Évolutive, Université Lyon 1, CNRS, UMR 5558, 69622 Villeurbanne cedex, France; [d]Department of Earth & Environmental Sciences & GeoBio-Center, Ludwig-Maximilians-Universität München, Munich 80333, Germany; [e]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125; [f]Department of Sustainable Agro-Ecosystems and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all' Adige 38010, Italy; [g]Centre for Biodiversity Theory and Modelling, USR CNRS 2936, Station d'Ecologie Expérimentale du CNRS, 09200 Moulis, France; [h]Département de Biochimie, Centre Robert-Cedergren, Université de Montréal, Montreal, QC, Canada H3C 3J7; and [i]Bayerische Staatssammlung für Paläontologie und Geologie, Munich 80333, Germany

Author contributions: D.P., W.P., M.D., R.F., O.R.-S., H.P., N.L., and G.W. wrote the paper.

The authors declare no conflict of interest.

[1]D.P. and W.P. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: davide.pisani@bristol.ac.uk or woerheide@lmu.de.



www.pnas.org/cgi/doi/10.1073/pnas.1525718113

1 Halanych KM, Whelan NV, Kocot KM, Kohn AB, Moroz LL (2016) Miscues misplace sponges. *Proc Natl Acad Sci USA* 113:E946–E947.
2 Pisani D, et al. (2015) Genomic data do not support comb jellies as the sister group to all other animals. *Proc Natl Acad Sci USA* 112(50):15402–15407.
3 Whelan NV, Kocot KM, Moroz LL, Halanych KM (2015) Error, signal, and the placement of Ctenophora sister to all other animals. *Proc Natl Acad Sci USA* 112(18): 5773–5778.
4 Ryan JF, et al.; NISC Comparative Sequencing Program (2013) The genome of the ctenophore *Mnemiopsis leidyi* and its implications for cell type evolution. *Science* 342(6164):1242592.
5 Moroz LL, et al. (2014) The ctenophore genome and the evolutionary origins of neural systems. *Nature* 510(7503):109–114.